ERCC

Performance of RNA Standards

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EXTERNAL RNA CONTROLS CONSORTIUM WORKSHOP

NIST

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GOAL:

To develop a set RNA standards that will provide a range of signal intensities and/ or gene expression ratios that mimics what is found in "normal" mammalian cells.

"Average" cell: Contains 25 pg total RNA and 2% or 0.5 pg mRNA

#mRNA Molecules/cell: 100,000-500,000

Sensitivity Definition: molecules mRNA per 100,000 or parts per million

Example: 25 ug RNA = 1 million cells or >10^{11} mRNA molecules. To spike in at conc of ~1 part per million use 100,000 molecules of standard

Testing Performance

Spikes: 0, 1, 10, 50, 100……1000 copies

Ratios: 50/1, 25/1, 10/1, 5/1, 2/1, 1/1

Questions:

Lowest signal (lowest copy number) detection?

Perhaps use absolute numbers only; 10^4, 10^5, 10^6, etc.

What is dynamic range expected? 10^0 to $10^?$

More spikes or ratios required? More the better or will more make QC, analysis, etc difficult?